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Changes of topsoil under *Fagus sylvatica* along a small latitudinal-altitudinal gradient

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ABSTRACT

This study evaluated soil properties along a small transect in the Apennines chain (central Italy). Using latitude and altitude as surrogates for temperature differences, three locations at different latitudes were selected for the study. At each location, two altitudes were selected (800 and 1000 m). The study was conducted by contrasting chemical and biochemical parameters [microbial biomass-C content, amount of CO_2 evolved during basal respiration (ΣCO_2–C), and potential enzyme activities] of topsoils (O and A horizons) supporting European beech (*Fagus sylvatica* L.) forests at different scales of investigation: horizons, altitude, and latitude. Along the topsoil, the trend of all investigated properties was unique to each O and A horizon according to its level of organic matter degradation, availability of substrates, and nutrients. Contrasting the altitudes, the difference of 1 °C of temperature between 800 and 1000 m significantly affected some of the chemical parameters and both microbial and enzyme activities mainly in the mineral horizons, although glucuronidase and phosphodiesterase activities appeared to be mainly controlled by TOC content. Generally, the different enzyme activities were more clearly highlighted when they were expressed per organic C unit, rather than on a soil mass basis. Latitudinal sensitivity was recorded for total N, ΣCO_2–C, and some enzyme activities, although no difference in mean annual air temperature occurred along the latitudinal transect. In this case, it is plausible that the summer-winter thermal excursion, which was lower for the southernmost location and increased going north, might have indirectly affected these parameters through changes in the plant-soil relationships, composition of the macro- and mesofauna population, and microbial activity. Considering all the investigated aspects, an increase of 1 °C in mean annual air temperature (in the range 9–10 °C) and/or the alteration of summer-winter thermal excursion might affect the actual soil-plant balance in temperate beech forests, with possible greater mineralization of the topsoil organic matter and a major release of CO_2 in the atmosphere.

1. Introduction

Climate is one of the main factors controlling vegetation, litter degradation, and soil formation. Several ecological consequences are attributed to increasing temperature, which modifies natural environments (Lindner et al., 2010), but the relationship between soil and vegetation under changing climate requires careful examination. According to Aerts (2006), climate can control litter degradation both directly and indirectly. Through changes in soil temperature and moisture, climate directly alters the rates of litter mass loss. Indeed, the high sensitivity of biological processes to temperature and water availability affects the plant community composition and growth, litter quality, and composition and activity of detritivore and decomposer communities (Strickland et al., 2015; Aerts et al., 2006). Consequently, it could be hypothesized that global warming will influence litter decomposition and soil morphology and functionality in the same way it affects species distribution shifting polewards in latitude and upwards in elevation (Corlett and Westcott, 2013; IPCC, 2007; Lenoir et al., 2008; Lindner et al., 2010; Peñuelas et al., 2007), with a re-arrangement of the ecological equilibria occurring between organic and mineral soil horizons.

Soil microbiota and its enzymatic pool play one of the main roles in

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the decomposition processes through physicochemical breakdown of plant and animal residues (Aerts, 2006; Kourtev et al., 2002). Thus, soil microbial activity is intimately linked to litter degradation, a fundamental process maintaining ecosystem functions. Plant biomass production is supported by organic matter mineralization and humification, which allow elemental cycling ensuring nutrients to plants (e.g., Mincheva et al., 2014; Swift et al., 1979; Wang et al., 2015). Soil microbial activity and enzyme activities are influenced by soil type, vegetation, litter quality, and environmental factors; also, enzyme activities can modify the substrate, and this may lead to microbial community succession (Sinsabaugh et al., 2002). Hence, enzyme activities have been often used as indicators of change in soil functions (e.g., Allen et al., 2011; Schloter et al., 2003), although it is problematic to use the sole enzyme activities as indicators of soil perturbations because enzymatic assays determine potential and not real enzyme activities (Nannipieri et al., 2012; Rao et al., 2014). It has been recognized that beyond biotic factors, atmospheric warming and precipitation patterns substantively influence soil enzyme activity (e.g., Burns et al., 2013; Steinweg et al., 2012; Wallenstein et al., 2011) and microbial substrate efficiency (Frey et al., 2013; Manzoni et al., 2012; Zhang et al., 2013). Burns et al. (2013) reported that the change of soil temperature, atmospheric CO_2 concentration, and frequent wetting-drying cycles affect enzyme activity, directly or through stimulation of plant growth and litter deposition.

To investigate the effect of temperature on soil morphology and on chemical and biochemical processes, a sampling strategy was designed to mimic temperature gradients by using latitude and altitude as proxies for temperature change (Vincent et al., 2014). Following this rational, on the Apennines chain (central Italy) we selected three mountain locations within 1° of latitude and, at each location, two sites at different altitude (800 and 1000 m). The six sites were similarly managed forests of European beech (*Fagus sylvatica* L.), the most diffused tree in the Mediterranean mountains. The small transect in altitude and latitude was consciously selected to evaluate the effect of 1 °C of temperature change between altitudes, being aware that the soil organic matter (SOM) content is controlled by temperature.

Specifically, the objective of this work was to establish the variability in chemical and biochemical characteristics of the O (organic, formed by decaying litter) and A (mineral) horizons, at three different detail levels: *i*) microscale, by contrasting soil horizons; *ii*) mesoscale, by contrasting soils at different altitudes; *iii*) macroscale, by contrasting soils at different latitudes. Altitude and latitude were used as surrogates of potential temperature changes, with assessments made on subsequent effects to changes in soil morphology, litter decomposition, and soil functionality. In doing so, we hypothesized that topsoil chemical and biochemical parameters would differ according to horizons, altitude, and latitude.

2. Materials and methods

2.1. Study sites and climatic features

Three study locations with similar lithology, physiography, vegetation, and forest management were selected along a south-to-north transect of 1° of latitudinal gradient in the central Apennines (Italy): Mount Terminillo (MT) (42°28′ N, 12°56′ E), Mount San Vicino (MV) (43°19′ N, 13°03′ E), and Mount Acuto (MA) (43°28′ N, 12° 41′ E) (Fig. 1). At the three locations the soil parent material was limestone and there was only a slight variation in the forest tree composition, with a greater presence of beech at MT and MA than at MV (Table S1 of Supplementary Materials). At each location, on the north-facing slope hosting a mesophylic European beech (*Fagus sylvatica* L.) coppice in conversion to timber forest, two sites were selected at ~800 and 1000 m above sea level (a.s.l.). The study sites were selected following a pedological survey to minimize the unavoidable differences in soil properties due to spatial variability. The climatic data were calculated

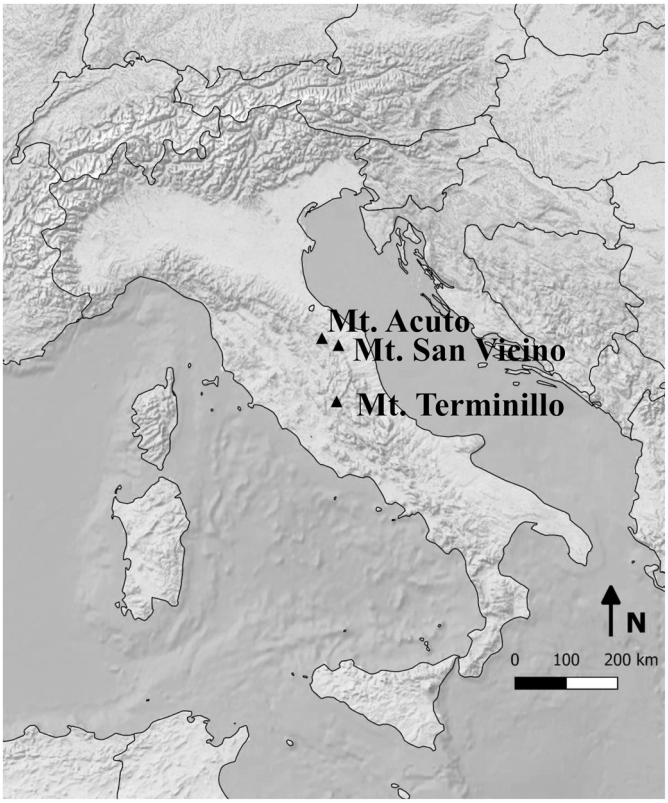

Fig. 1. Map of Italy indicating the localization of the study sites.

from > 50 years of consecutive records recovered from two official national stations: 1) Centro Appenninico "C. Jucci" (2015), based at 1700 m a.s.l. on MT (approximate distance of the climatic station from MT study site was 1 km), with climatic records from 1956 to 2014; and 2) Marche Region Civil Protection Department (Italy), which published the climatic records of the 1950–2000 series in Spina et al. (2006) and Amici and Spina (2002) (approximate distance of the climatic station from MA and MV study sites was 2 and 13 km, respectively). The first series was used for the MT location, while the second for both MV and MA. The reconstructed climatic conditions reported in Table S1 of Supplementary Materials indicate that the three locations had a mean annual precipitation ranging from 825 to 1430 mm, which was similar at both altitudes. Along the small latitudinal gradient considered (1° of latitude), there was no difference in terms of mean annual air temperature (MAAT), although the northernmost location (MA) was characterized by a higher mean summer air temperature of 1.2 °C and a lower mean winter air temperature of 1.7 °C with respect to the southernmost location (MT). At the three locations, the two sites at 800 and 1000 m a.s.l. featured MAAT of 10 °C and 9 °C, respectively.

2.2. Soil sampling

The samples were collected in March/April 2015. This period of sampling was chosen since the European beech is a dormant species, and in this period both plant and microbial community activities at these altitudes are more stable than in dynamic seasons like spring and summer (De Feudis et al., 2016, 2017). To assess the spatial variability of the litter and its properties, five to six pits were dug at each site. On the basis of these observations, two 50-m^2 surface plots representative of area conditions were selected at both altitudes and, in each plot, a topsoil profile was opened at 50–60 cm downslope the stem of a beech. Minimum distance between the two profiles was 8 m, and the maximum was 14 m. The profiles were morphologically described per Schoeneberger et al. (2012) and Baize and Girard (2008) for the O horizons (general characteristics of

these horizons are reported in Section S2 of Supplementary Materials), and by Schoeneberger et al. (2012) for the mineral horizons.

A 40 × 40 cm sampling frame was used to collect both O and A horizons. Depending on the horizon thickness, the sampled amount for the O horizons ranged from 270 to 910 g (air-dry weight); for the A horizons, the whole sample was homogenized in the field and an aliquot of ~3 kg was collected. In the field, all the samples were stored in a portable refrigerator. Once in the laboratory, a quarter of each sample was stored at 4 °C for biochemical analyses, while the rest was air-dried at room temperature. About one half of each dried O sample was stored at room temperature, while the other half was triturated until it passed through a 1 mm sieve to ensure homogeneity of the samples and representativeness of the aliquots to be used for analyses. The A horizon samples were sieved past 2 mm and the fine earth was deprived of the visible roots under a 2:2 magnifying lens.

2.3. Chemical analysis

Soil pH was determined potentiometrically in water (pH_{H2O}) with a solid:liquid (w:v) ratio of 1:8 and 1:2.5 for O and A horizons, respectively. Total organic C (TOC) content was estimated by K-dichromate digestion, heating the suspension at 180 °C for 30 min (Nelson and Sommers, 1996), while total N (TN) was determined by dry combustion analyzer (EA-1110, Carlo Erba Instruments, Milan, Italy). Pyrophosphate extractable organic C (C_p), which estimates labile organic matter plus free and metal-complexed humic substances (e.g., Bakina et al., 2017), was determined per Bascomb (1968). For details on this method see Section S3 of Supplementary Materials. Summarily, 2 g aliquots of non-triturated samples were used for the OLn and OLv horizons (the representativeness of the sample was ensured by considering the percentage of each organic debris composing the horizons), and 1 g for OH and A horizons, which are naturally more homogeneous than OL horizons.

2.4. Biochemical analysis (microbial biomass, basal respiration, and enzyme activities)

To assess the amount of microbial biomass-C (Cmic) by fumigation–extraction (Vance et al., 1987), two sets of samples (for the organic horizons non-triturated samples were used) were maintained for 50 days at 25 °C and at 50% of their total water holding capacity. Thus, one set of samples was exposed to alcohol-free chloroform ($CHCl_3$) vapor in a vacuum desiccator at 25 °C for 24 h. Then, $CHCl_3$ was removed from the fumigated soils by repeated evacuations. Both the fumigated and not-fumigated samples were shaken for 30 min with 0.5 M K_2SO_4 (solid:liquid ratio 1:4). The extracted organic C was measured by oxidation with K-dichromate and a subsequent back-titration of the unreduced dichromate. During the incubation period, basal respiration was measured by alkali (1 M NaOH solution) absorption of the CO_2 developed and titration of the residual OH^- with a standardized HCl solution. Basal respiration was expressed as the cumulative amount of CO_2–C that evolved during the 50-days experiment (ΣCO_2–C).

A set of 14 enzyme activities was quantified according to Cowie et al. (2013). As mineral and organic samples were compared, an extraction method was employed to avoid problems of imperfect slurry production [generally preferred by DeForest, 2009, Rao et al., 2014, and Wang et al., 2016] as in the case of non-triturated OLn and OLv samples. The specific role of enzymes in natural cycles is given in Table S4 of Supplementary Materials. Briefly, 150 mg of non-triturated aliquots from O horizons and 250 mg of sample from A horizons stored for < 10 days at 4 °C were placed in a 2-ml Eppendorf tube with glass beads and 1.2 ml of 50 mM tris–HCl solution at pH 7.0, containing 2% lysozyme as a desorbing protein. The tube was subjected to bead-beating (3 min, 30 strokes s^{-1}) using a Retsch MM400 mill, then centrifuged for 5 min at 20,000*g*. Enzyme activity was assayed fluorometrically in microplates using 4-methyl-umbelliferyl and 7-amino-4-methyl coumarine conjugated surrogate substrates (Sigma, St. Louis, MO, USA). The activities of arylsulfatase, chitinase, glucuronidase, α-glucosidase, β-glucosidase, cellulase, xylosidase, acid phosphomonoesterase, and inositol-phosphatase were determined in 200 mM MES (morpholineptansulfonic acid) solution at pH 5.8; the activities of leucine aminopeptidase acetate-esterase, pyrophosphatase-phosphodiesterase, and phosphodiesterase were determined in 100 mM tris–HCl solution at pH 7.5; alkaline phosphomonoesterase activity was determined in 100 mM tris–HCl solution at pH 9.0. Since SOM content affects enzyme activities, these latter were expressed on a soil mass basis (nanomoles of substrate·g^{-1} of dry soil·h^{-1}) to appraise the ecosystem-level measure, but they were also normalized per unit of organic C (nanomoles of substrate·g^{-1} of organic C·h^{-1}) to appraise the nutritional status of SOM (Boerner et al., 2005).

2.5. Statistical analysis

Each sample of the 12 profiles (3 locations × 2 altitudes × 2 profiles) was analyzed in duplicate. The two values obtained were averaged to calculate the analytical mean for each horizon. For the calculations relative to altitude and latitude, the organic and mineral horizons were maintained separately. When the same genetic horizon was present with different morphological features (e.g., OLv1 and OLv2, or A1 and A2), a weighted mean for each property was calculated based upon its respective thickness. The mean and relative standard deviation were then calculated for horizons (n = 12 for OLn, OLv, and A; n = 8 for OH), altitudes (n = 16 for organic horizons, n = 6 for mineral horizons), and locations (n = 12 for the organic horizons of MT and MA and n = 8 for the organic horizons of MV; n = 4 for the mineral horizons). The n difference among horizons and among latitudes was due to the absence of OH horizons in the MV profiles (Table S1). For the topsoil thickness, mean and standard deviation were calculated for both organic and mineral horizons by altitude (n = 6) and latitude (n = 6).

An overall view of variations and correlations of soil chemical and biochemical properties (with the enzyme activities expressed on a soil mass basis) as function of horizon, altitude, and latitude was assessed by non-metric multidimensional scaling (NMDS) analysis by using the R package “vegan” with the dissimilarity matrix calculated by the Gower's distance. The fitted vectors are arrows that represent the direction of the properties and their length is proportional to the strength of the properties (Oksanen, 2015). Before the NMDS, data were standardized by subtracting the mean and dividing by the standard deviation. To assess the relationship between enzyme activities (arylsulphatase and pyrophosphatase-phosphodiesterase) and soil properties (pH, TOC, and Cp), a simple linear correlation was performed.

Analysis of variance (ANOVA) was carried out to test the effect of altitude and latitude on the thickness of O and A horizons, and of horizons, altitude, and latitude on chemical and biochemical properties in order to estimate the significance of each property. To apply the ANOVA, we previously verified the normal distribution of the data and successively the equal variances (See Table S5 of Supplementary Materials). When data were not parametric (non-normal distribution and heterogeneity of the variance), each numerical variable was transformed by the Box and Cox (1964) procedure. The improvement of the assumption to normality and homoscedasticity was verified on residuals by the Shapiro-Wilk statistical test (“stats” package) and by Levene's test (“car” package) both at 5% significance level. When normality was not respected, the Kruskal-Wallis test was applied to assess if the differences were significant. In case of heteroscedasticity of the transformed data, the Welch one-way ANOVA test was performed. The unbalanced design of the dataset for organic horizons due to the absence of OH in MV was considered applying a Type II ANOVA. ANOVA tests were deemed significant when $P \leq 0.05$; differences between means were compared using a post-hoc Tukey's Honest Significant Difference (HSD) test with $P \leq 0.05$.

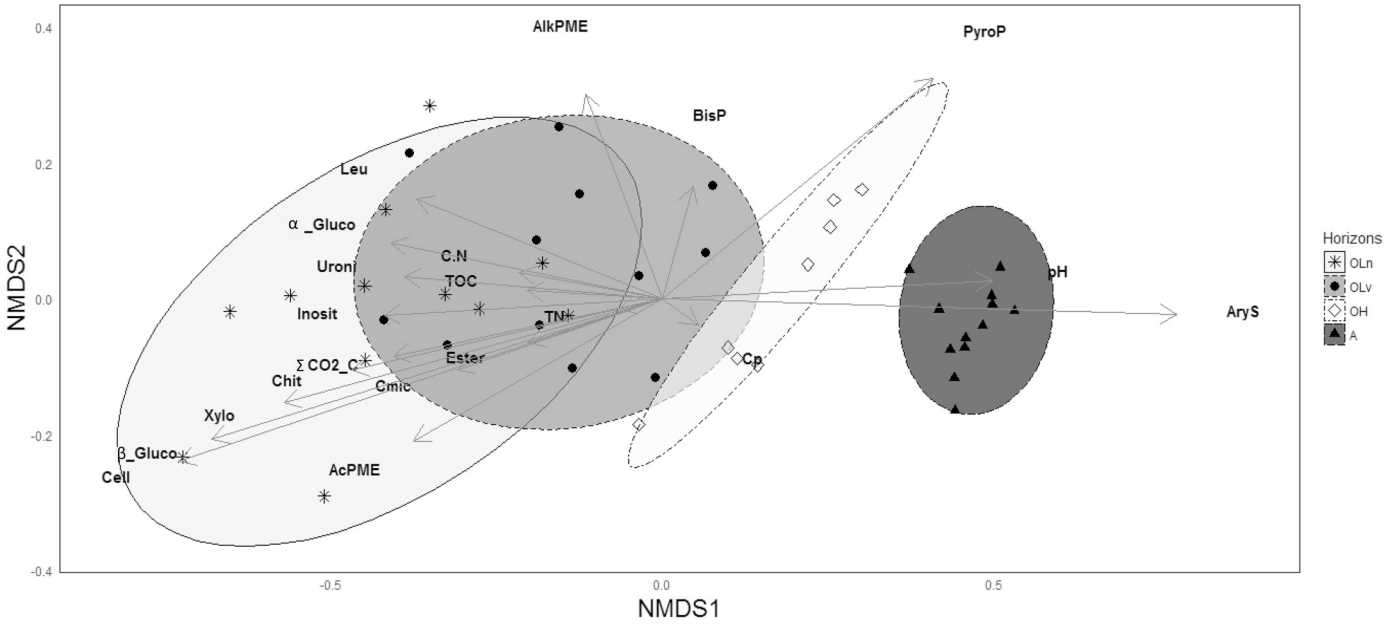

Fig. 2. Variation of topsoil chemical and biochemical properties as obtained by the NMDS analysis according to horizons. Chemical and biochemical properties: ΣCO_2–C = amount of CO_2 evolved during basal respiration experiments; C/N = C/N ratio; Cmic = microbial biomass carbon; C_p = Na-pyrophosphate extractable organic C; TN = total N; TOC = total organic C. Enzymes activities: AcPME = acid phosphomonoesterase activity; AlkPME = alkaline phosphomonoesterase activity; AryS = arylsulfatase activity; BisP = phosphodiesterase activity; Cell = cellulase activity; Chit = chitinase activity; Ester = acetate-esterase activity; Inosit = inositol-phosphatase activity; Leu = leucine-aminopeptidase activity; PyroP = pyrophosphatase-phosphodiesterase activity; Uroni = glucuronidase activity; Xylo = xylosidase activity; α-Gluco = α-glucosidase activity; β-Gluco = β-glucosidase activity.

Table 1
P and R^2 values of fitted vectors in the NMDS analysis of chemical and biochemical properties of the organic (O) and mineral (A) horizons of soils under European beech (*Fagus sylvatica* L.) forest from central Apennines (Italy) within 1° of latitude.

	pH	TOC	TN	C/N	Cp	Cmic	ΣCO_2–C	AryS	Chit	Leu	Uroni	α-Gluco	β-Gluco	Cell	Xylo	AcPME	BisP	PyroP	AlkPME	Inosit	Ester
NMDS by horizons																					
P	0.00	0.00	0.00	0.00	0.29	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	***	***	***	***	ns	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
R^2	0.55	0.77	0.35	0.75	0.06	0.39	0.90	0.75	0.90	0.67	0.76	0.87	0.80	0.71	0.88	0.59	0.53	0.56	0.87	0.83	0.69
NMDS by organic horizons (O)																					
P	0.00	0.00	0.00	0.00	0.48	0.25	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	***	***	***	***	ns	ns	***	***	***	***	***	***	***	***	***	**	***	***	***	***	***
R^2	0.44	0.64	0.71	0.62	0.05	0.09	0.75	0.68	0.78	0.48	0.57	0.77	0.74	0.61	0.78	0.39	0.62	0.82	0.47	0.77	0.67
NMDS by mineral horizon (A)																					
P	0.00	0.01	0.05	0.00	0.01	0.02	0.13	0.07	0.02	0.00	0.01	0.02	0.00	0.06	0.00	0.00	0.00	0.01	0.02	0.09	0.02
	**	*	*	***	*	*	ns	ns	*	**	*	*	**	ns	**	**	**	**	*	ns	*
R^2	0.58	0.63	0.52	0.74	0.64	0.57	0.37	0.48	0.53	0.70	0.66	0.59	0.77	0.47	0.82	0.76	0.73	0.65	0.51	0.42	0.57

Level of significance: ns = not significant.
TOC = total organic carbon; TN = total nitrogen; C/N carbon/nitrogen ratio; C_p = Na-pyrophosphate extractable organic carbon; Cmic = microbial biomass carbon; (ΣCO_2–C) = amount of CO_2 evolved during basal respiration experiments; AryS = arylsulfatase activity; Chit = chitinase activity; Leu = leucine-aminopeptidase activity; Uroni = glucuronidase activity; α-Gluco = α-glucosidase activity; β-Gluco = β-glucosidase activity; Cell = cellulase activity; Xylo = xylosidase activity; AcPME = acid phosphomonoesterase activity; BisP = phosphodiesterase activity; PyroP = pyrophosphatase-phosphodiesterase activity; AlkPME = alkaline phosphomonoesterase activity; Inosit = inositol-phosphatase activity; Ester = acetate-esterase activity.
* = $P \leq 0.05$.
** = $P \leq 0.01$.
*** = $P \leq 0.001$.

3. Results

3.1. Soil general features

Topsoil profiles showed similar morphologies among profiles, altitudes, and latitudes (Table S1). The main differences were a greater sequence of both O and A horizons in the MT soils with respect to the others. Thus, the A horizons showed a prevailing sub-angular blocky structure in the MT soils, a crumb structure in the MA soils, and an intermediate state of aggregation in the MV soils. Soil drainage increased from MT to MV and, then, to MA, possibly in function of the soil structure. The small macro- and mesofauna abounded in the OLv and OH horizons of all soils. Soils were classified as Mollisols at MT, and as Mollisols and Inceptisols at MV and MA. At the moment of sampling, the water content of the samples ranged from 42 to 71% (w/w) in the O horizons, and from 37 to 59% (w/w) in the A horizons (data not shown).

3.2. Microscale level: horizon effect

The result of the NMDS analysis (stress = 0.07) is reported in Fig. 2,

Table 2
Results of ANOVA showing the influence of horizons, altitude, and latitude on topsoil thickness and on chemical and biochemical properties of the organic (O) and mineral (A) horizons of soils under European beech (*Fagus sylvatica* L.) forest from central Apennines (Italy) within 1° of latitude.

	Horizons		Altitude				Latitude			
			O horizons		A horizon		O horizons		A horizon	
	F value	Signif.	*F* value	Signif.	*F* value	Signif.	*F* value	Signif.	*F* value	Signif.
Topsoil thickness	–	–	0.25	ns	2.36	ns	1.46	ns	2.18	ns
Chemical and biochemical properties										
pH	8.82	***	0.02	ns	1.69	ns	2.39	ns	3.57	ns
TOC	220.21	***	5.79	*	2.77	ns	1.81	ns	2.70	ns
TN	34.66	***	7.94	**	12.44	**	7.73	**	1.01	ns
C/N	59.42	***	0.53	ns	15.14	**	1.52	ns	0.17	ns
C_p	3.90	*	0.15	ns	6.84	*	0.15	ns	1.40	ns
Cmic	165.93	***	0.02	ns	9.11	*	1.44	ns	0.38	ns
ΣCO_2–C	73.22	***	0.04	ns	0.26	ns	6.04	*	1.57	ns
Enzyme activities expressed on a soil mass basis										
AryS	69.84	***	1.23	ns	0.65	ns	2.68	ns	2.44	ns
Chit	112.18	***	0.01	ns	3.43	ns	1.01	ns	0.49	ns
Leu	62.00	***	0.20	ns	0.99	ns	0.00	ns	1.41	ns
Uroni	28.19	***	0.37	ns	7.51	*	1.84	ns	0.57	ns
α-Gluco	34.01	***	0.01	ns	2.23	ns	0.53	ns	1.83	ns
β-Gluco	104.75	***	0.38	ns	4.00	ns	1.46	ns	1.18	ns
Cell	35.92	***	0.49	ns	3.68	ns	1.36	ns	1.24	ns
Xylo	114.46	***	0.35	ns	4.65	ns	1.72	ns	2.91	ns
AcPME	29.69	***	0.04	ns	4.83	ns	1.05	ns	1.04	ns
BisP	2.50	ns	0.15	ns	8.42	*	4.99	*	0.35	ns
PyroP	3.52	*	0.39	ns	0.09	ns	12.16	***	2.07	ns
AlkPME	12.41	***	2.20	ns	1.01	ns	8.79	*	0.15	ns
Inosit	89.72	***	0.56	ns	0.05	ns	0.38	ns	3.34	ns
Ester	19.84	***	0.02	ns	3.83	ns	2.44	ns	8.02	**
Enzyme activities relative to organic C unit										
AryS	118.73	***	0.37	ns	6.17	*	2.72	ns	0.70	ns
Chit	37.00	***	0.50	ns	12.24	**	0.76	ns	0.15	ns
Leu	13.23	***	1.20	ns	11.33	**	0.05	ns	0.81	ns
Uroni	7.57	**	1.65	ns	9.60	*	1.24	ns	1.23	ns
α-Gluco	16.60	***	0.24	ns	7.55	**	0.27	ns	0.79	ns
β-Gluco	45.86	***	0.91	ns	12.96	**	1.31	ns	1.06	ns
Cell	35.03	***	1.01	ns	7.87	*	1.21	ns	0.84	ns
Xylo	45.65	***	1.13	ns	17.70	**	1.47	ns	1.42	ns
AcPME	12.78	***	0.91	ns	13.68	**	0.31	ns	1.17	ns
BisP	76.47	***	0.66	ns	0.09	ns	8.38	**	2.09	ns
PyroP	73.97	***	0.02	ns	2.42	ns	11.69	***	0.92	ns
AlkPME	17.38	***	0.19	ns	1.66	ns	7.07	**	1.71	ns
Inosit	13.35	***	2.50	ns	5.04	*	0.10	ns	1.98	ns
Ester	21.62	***	1.76	ns	8.43	*	1.39	ns	3.09	ns

Level of significance: ns = not significant.
TOC = total organic carbon; TN = total nitrogen; C/N = carbon/nitrogen ratio; C_p = Na-pyrophosphate extractable organic carbon; Cmic = microbial biomass carbon; ΣCO_2–C = amount of CO_2 evolved during basal respiration experiments; AryS = arylsulfatase activity; Chit = chitinase activity; Leu = leucine-aminopeptidase activity Uroni = glucuronidase activity; α-Gluco = α-glucosidase activity; β-Gluco = β-glucosidase activity; Cell = cellulase activity; Xylo = xylosidase activity; AcPME = acid phosphomonoesterase activity; BisP = phosphodiesterase activity; PyroP = pyrophosphatase-phosphodiesterase activity; AlkPME = alkaline phosphomonoesterase activity; Inosit = inositol-phosphatase activity; Ester = acetate-esterase activity.
* = $P \leq 0.05$.
** = $P \leq 0.01$.
*** = $P \leq 0.001$.

while *P* and R^2 values of the fitted vectors of the NMDS analysis are in Table 1. These results showed the occurrence of relationships among all the considered soil properties, indicating that horizons had distinct chemical and biochemical properties. The clusters of OLn and OLv horizons partially overlapped, indicating a slight difference between these two types of horizon for the considered properties, which largely depended on the state of SOM degradation. The OH horizons differed from the OLn ones, and showed only a slight overlap with the OLv cluster. However, the O horizons were frankly different from the A horizons.

The specific behavior of chemical and biochemical properties along the topsoil was analyzed singularly by ANOVA (Table 2), which showed that the horizon effect was significant for all tested properties with the only exception being phosphodiesterase activity expressed on a soil mass basis. In particular, pH followed an increasing trend with depth, TOC content and C/N ratio decreased from OLn to A horizons, while TN content was constant from OLn to OH horizons with a sharp decrease in the A horizons (Table 3). C_p was the highest in the OH horizons (33.3 g kg^{-1}) and the lowest in the A horizons (17.6 g kg^{-1}). The Cmic content and the ΣCO_2-C released during basal respiration decreased from the OLn to the A horizon (Table 3). When expressed on organic C unit, the horizon effect was significant for all the enzyme activities (Table 2). Generally, the trend with depth was the same when the enzyme activities were expressed on a soil-mass basis or organic C content basis (Tables 4 and 5). In particular, in both cases, arylsulfatase activity increased with depth, while chitinase, leucine-aminopeptidase, glucuronidase, α-glucosidase, β-glucosidase, cellulase, xylosidase, inositol-phosphatase, and acetate-esterase activities substantially followed the

Table 3
Topsoil thickness and pH, total organic carbon (TOC), Na-pyrophosphate extractable organic C (C_p), total nitrogen (TN), carbon/nitrogen ratio (C/N), microbial biomass carbon (Cmic), and amount of CO_2 evolved during basal respiration experiments (ΣCO_2–C) of the organic horizons (O) and the first mineral horizon (A) at a micro- (horizon), meso- (altitude), and macro- (latitude) scale for mountain soils under European beech (*Fagus sylvatica* L.) forest from central Apennines (Italy) within 1° of latitude. The numbers in parentheses are the standard deviations. Within each variable (horizons, altitude, and latitude), column with different letters have means values that significantly differ for $P \leq 0.05$ for the Tukey's post-hoc test; for the altitude and latitude variables, lowercase letters refer to O horizons, capital letters to A horizons.

	Thickness		pH		TOC		TN		C/N		C_p		Cmic		ΣCO_2–C	
	O	A	O	A	O	A	O	A	O	A	O	A	O	A	O	A
	cm				g kg^{-1}		g kg^{-1}				g kg^{-1}		mg kg^{-1}		mg kg^{-1}	
Horizons																
OLn	–		**6.18^{c}**		**506.2^{a}**		**14.5^{a}**		**35.0^{a}**		**25.4^{ab}**		**282.6^{a}**		**20417.6^{a}**	
			(0.23)		**(64.2)**		**(3.1)**		**(20.8)**		**(14.7)**		**(148.2)**		**(7182.6)**	
OLv	–		**6.51^{bc}**		**496.4^{a}**		**16.0^{a}**		**31.1^{a}**		**29.7^{ab}**		**207.5^{ab}**		**14410.7^{a}**	
			(0.33)		**(58.6)**		**(2.1)**		**(28.2)**		**(13.5)**		**(163.8)**		**(5742.2)**	
OH	–		**6.91^{ab}**		**409.1^{b}**		**17.8^{a}**		**23.0^{b}**		**33.3^{a}**		**166.9^{b}**		**8273.0^{b}**	
			(0.50)		**(63.1)**		**(3.8)**		**(16.6)**		**(6.2)**		**(203.8)**		**(3697.8)**	
A	–		**7.18^{a}**		**79.3^{c}**		**6.0^{c}**		**13.3^{c}**		**17.6^{b}**		**0.2^{c}**		**943.9^{c}**	
			(0.70)		**(36.0)**		**(3.0)**		**(12.2)**		**(5.4)**		**(0.1)**		**(577.2)**	
Altitude																
800 m	7.7	9.5	6.49	6.90	**449.2^{b}**	63.5	**14.5^{b}**	**4.0^{b}**	31.1	**15.9^{a}**	30.1	**14.5^{b}**	242.3	**0.1^{b}**	15562.7	1174.3
	(1.5)	(4.5)	(0.43)	(0.82)	**(75.3)**	(14.1)	**(3.0)**	**(1.3)**	(25.1)	**(10.8)**	(14.8)	**(3.2)**	(209.5)	**(0.0)**	(8517.6)	(756.6)
1000 m	10.7	17.3	6.49	7.45	**507.3^{a}**	95.0	**17.3^{a}**	**7.9^{a}**	29.4	**12.0^{b}**	27.9	**20.8^{a}**	208.7	**0.2^{a}**	14694.9	713.5
	(5.6)	(9.9)	(0.47)	(0.48)	**(58.0)**	(45.4)	**(2.7)**	**(2.9)**	(21.5)	**(15.7)**	(10.4)	**(5.5)**	(124.0)	**(0.0)**	(6580.2)	(182.2)
Latitude																
Mount Terminillo	12.3	19.8	6.67	7.66	444.6	77.9	**13.8^{b}**	6.1	32.2	12.8	28.6	18.7	166.1	0.2	**12446.3^{b}**	1324.9
	(5.2)	(8.7)	(0.52)	(0.48)	(90.6)	(7.7)	**(2.6)**	(2.0)	(34.8)	(3.9)	(11.1)	(6.3)	(134.3)	(0.1)	**(7233.5)**	(696.3)
Mount San Vicino	7.0	8.0	6.53	6.64	498.3	103.0	**15.7^{ab}**	7.5	31.7	13.7	27.4	20.0	292.4	0.2	**11759.2^{b}**	684.1
	(1.6)	(1.8)	(0.40)	(0.60)	(36.4)	(55.5)	**(1.4)**	(4.4)	(26.0)	(12.6)	(14.8)	(6.0)	(179.5)	(0.1)	**(3049.8)**	(255.0)
Mount Acuto	8.3	12.5	6.28	7.23	498.6	56.9	**18.0^{a}**	4.3	27.7	13.2	30.4	14.2	240.4	0.1	**20057.7^{a}**	822.7^{a}
	(3.9)	(9.3)	(0.30)	(0.72)	(61.2)	(13.8)	**(3.1)**	(1.3)	(19.7)	(10.6)	(13.5)	(2.5)	(189.0)	(0.0)	**(7594.2)**	(607.2)

Data with statistically significant differences are highlighted using bold text.

opposite trend. Conversely, four enzyme activities displayed different trends depending on the manner in which they were expressed. This was the case of acid phosphomonoesterase, phosphodiesterase, pyrophosphatase-phosphodiesterase, and alkaline phosphomonoesterase, which showed the highest activities in one or more of the O horizons when expressed on a soil mass basis, while the highest activities in the A horizon were expressed on the organic C unit.

3.3. Mesoscale level: altitude effect

On the basis of the NMDS analysis (stress = 0.11 and 0.06 for organic and mineral horizons, respectively), altitude did not affect the general pattern of chemical and biological properties of the O horizons (all properties of the soils at 800 and 1000 m a.s.l. substantially clustered together). However, it had a remarkable effect on the characteristics of the A horizons (Fig. 3a, b). Thus, the contents of TOC and TN in the organic horizons and of TN, Cp and Cmic in the mineral ones were significantly greater at 1000 m than at 800 m. The C/N ratio of the A horizons was higher at the lower altitude (Tables 2 and 3). The different altitude appeared to not influence the enzyme activities of the O horizons, whether they were expressed on a soil mass basis or organic C unit (Table 2). Conversely in the underlying A horizons, altitude affected the enzyme activities especially when they were normalized per unit of organic C content. Indeed, when the enzyme activities were expressed on a soil mass basis, only glucuronidase and phosphodiesterase activities were significantly greater at 800 m than at 1000 m, but when they were normalized per unit of organic C content, 11 of 14 enzyme activities appeared to be affected by altitude (Table 2); the greatest activities occurred at 800 m (Tables 4 and 5). Although the cumulative thickness of the topsoil (O + A horizons) was 17.2 cm (standard deviation 4.4) at 800 m and 28.0 cm (standard deviation 13.8) at 1000 m (Table S1), altitude did not significantly affect either the topsoil thickness or the singular thickness of the horizons (Table 2).

3.4. Macroscale level: latitude effect

The NMDS analysis (stress = 0.11 and 0.06 for organic and mineral horizons, respectively) indicated that the difference of 1° latitude with no MAAT variation did not induce a generalized change in the extent of the considered properties for both O and A horizons (Fig. 4). However, the clustering between MT and MA A horizons appeared separated (Fig. 4b). A significant latitude effect occurred in the O horizons for TN content, ΣCO_2–C, and three of 14 enzyme activities, expressed on both a soil mass basis and organic C unit (Table 2). Total N and ΣCO_2–C decreased from the northernmost location (MA) to the southernmost location (MT) (Table 3), while the phosphodiesterase, pyrophosphatase-phosphodiesterase, and alkaline phosphomonoesterase activities displayed the opposite trend (Table 4). The topsoil cumulative thickness (O + A horizons) was 32.1 cm (standard deviation 13.0) at MT, 15.0 cm (standard deviation 3.4) at MV, and 20.8 cm (standard deviation 9.4) at MA (Table S1). In the case of altitude, latitude did not significantly affect either the topsoil thickness or the singular thickness of the O or A horizons (Tables 2 and 3).

4. Discussion

4.1. Microscale level: horizon effect

The different degradation stages of the litter floor induced a stratification recognizable by observation (from OLn to A horizons), and influenced both soil chemical and biochemical properties (e.g., Baldrian, 2014; Šnajdr et al., 2008). The NMDS analysis conducted for all tested properties showed that the differences in chemical and biochemical properties of the topsoil were peculiar for each horizon according to degradation levels of the organic residues.

Higher TOC content in the upper OLn horizons was due to scarce degradation of fresh residues (Wittmann et al., 2004), although TOC

Table 4
Soil enzyme activities expressed on a soil mass basis of the organic (O) and mineral (A) horizons at the micro- (horizon), meso- (altitude), and macro- (latitude) scale for soils under European beech (*Fagus sylvatica* L.) forest from central Apennines (Italy) within 1° of latitude. The numbers in parentheses are the standard deviations. Within each variable (horizons, altitude, and latitude), column with different letters have means values that significantly differ for $P \leq 0.05$ for the Tukey's post-hoc test; for the altitude and latitude variables, lowercase letters refer to O horizons, capital letters to A horizons.

	AryS		Chit		Leu		Uroni		α-Gluco		β-Gluco		Cell		Xylo		AcPME		BisP		PyroP		AlkPME		Inosit		Ester	
	O	A	O	A	O	A	O	A	O	A	O	A	O	A	O	A	O	A	O	A	O	A	O	A	O	A	O	A
	nanomoles of 4-methylumbelliferone (7-amino-4-methyl coumarine) • g^{-1} dry soil • $hour^{-1}$																											
Horizons																												
OLn	**8.0^{c}**		**216.1^{a}**		**372.5^{a}**		**5.2^{a}**		**10.6^{a}**		**621.7^{a}**		**97.3^{a}**		**63.4^{a}**		**434.1^{a}**		**51.4**		**19.4^{b}**		**389.7^{a}**		**36.0^{a}**		**3742.5^{a}**	
	(4.6)		**(68.3)**		**(129.4)**		**(1.6)**		**(2.5)**		**(322.7)**		**(67.5)**		**(26.0)**		**(305.4)**		**(14.0)**		**(13.1)**		**(173.6)**		**(10.6)**		**(794.3)**	
OLv	**13.0^{c}**		**120.9^{b}**		**376.9^{a}**		**3.9^{a}**		**7.9^{a}**		**215.8^{b}**		**31.2^{b}**		**29.6^{b}**		**283.5^{a}**		**45.6**		**35.2^{ab}**		**377.7^{a}**		**24.8^{b}**		**3129.4^{a}**	
	(8.9)		**(48.0)**		**(234.8)**		**(2.3)**		**(3.8)**		**(172.4)**		**(23.2)**		**(13.3)**		**(106.7)**		**(11.1)**		**(24.0)**		**(135.4)**		**(9.2)**		**(847.0)**	
OH	**28.1^{b}**		**48.4^{c}**		**98.7^{b}**		**1.7^{b}**		**2.0^{b}**		**33.9^{c}**		**3.4^{c}**		**6.8^{c}**		**159.3^{b}**		**37.2**		**45.2^{a}**		**331.2^{a}**		**9.1^{c}**		**1680.8^{b}**	
	(21.2)		**(24.9)**		**(47.9)**		**(1.6)**		**(1.3)**		**(26.4)**		**(2.9)**		**(4.5)**		**(56.6)**		**(13.1)**		**(34.8)**		**(107.3)**		**(2.8)**		**(894.0)**	
A	**65.9^{a}**		**5.1^{d}**		**24.8^{c}**		**0.4^{c}**		**0.6^{b}**		**4.4^{d}**		**0.2^{d}**		**1.0^{d}**		**92.0^{c}**		**41.5**		**33.1^{a}**		**158.1^{b}**		**4.7^{d}**		**1498.9^{b}**	
	(11.4)		**(4.5)**		**(12.4)**		**(0.4)**		**(0.4)**		**(1.7)**		**(0.2)**		**(0.6)**		**(34.9)**		**(11.3)**		**(16.8)**		**(50.1)**		**(1.2)**		**(838.0)**	
Altitude																												
800 m	13.1		138.5		304.7		3.9		7.1		349.9		50.		38.8		347.5		44.4		30.1		335.1		26.8		3023.2	
	(12.7)		(80.1)		(151.4)		(1.8)		(3.7)		(353.4)		(57.7)		(29.9)		(301.2)		(10.8)		(28.4)		(126.9)		(14.0)		(972.2)	
	68.5		6.8		26.1		**0.5^{A}**		0.7		5.3		0.3		1.3		109.0		**34.1^{B}**		33.8		169.1		4.8		1916.7	
	(13.1)		(5.0)		(5.3)		**(0.3)**		(0.1)		(1.4)		(0.2)		(0.4)		(32.5)		**(11.5)**		(17.7)		(54.3)		(0.7)		(887.3)	
1000 m	16.7		138.4		306.7		3.8		7.8		295.2		47.4		34.3		270.3		47.0		33.4		406.1		23.4		2971.1	
	(15.7)		(91.5)		(247.7)		(2.8)		(5.1)		(311.7)		(60.3)		(28.5)		(96.5)		(16.1)		(23.0)		(152.6)		(13.3)		(1340.8)	
	63.2		3.5		23.4		**0.2^{B}**		0.5		3.6		0.1		0.7		75.1		**48.8^{A}**		32.5		147.1		4.6		1081.2	
	(9.9)		(3.6)		(17.5)		**(0.4)**		(0.6)		(1.7)		(0.2)		(0.7)		(30.5)		**(4.2)**		(17.6)		(47.9)		(1.6)		(581.9)	
Latitude																												
Mount Terminillo	21.6		119.9		345.0		3.0		6.7		257.4		35.1		27.3		241.3		**51.5^{a}**		**47.0^{a}**		**429.6^{a}**		22.4		2425.5	
	(20.3)		(79.5)		(270.2)		(2.3)		(5.0)		(290.9)		(41.5)		(25.9)		(89.3)		**(8.5)**		**(29.3)**		**(70.8)**		(12.4)		(1141.4)	
	71.2		7.0		19.3		0.3		0.4		3.5		0.1		0.6		74.1		46.0		40.4		156.9		4.0		**784.2^{B}**	
	(10.9)		(6.6)		(8.1)		(0.4)		(0.3)		(1.3)		(0.2)		(0.5)		(25.8)		(6.8)		(17.8)		(54.6)		(1.3)		**(421.2)**	
Mount	14.5		117.3		267.6		3.9		7.7		205.6		31.1		34.5		286.8		**49.3^{ab}**		**35.0^{b}**		**405.2^{ab}**		26.9		3198.0	
San Vicino	(8.4)		(46.0)		(85.9)		(1.7)		(3.2)		(133.3)		(22.0)		(14.6)		(72.8)		**(16.7)**		**(23.6)**		**(210.8)**		(11.9)		(715.7)	
	69.6		4.8		33.2		0.4		1.0		4.9		0.3		1.4		103.3		39.3		36.1		175.7		5.8		**2305.9^{A}**	
	(10.3)		(4.0)		(15.7)		(0.5)		(0.4)		(1.0)		(0.1)		(0.4)		(18.9)		(13.9)		(22.1)		(69.7)		(1.0)		**(767.9)**	
Mount Acuto	8.5		171.1		291.8		4.7		8.0		465.6		74.9		47.2		391.3		**37.5^{b}**		**14.3^{b}**		**288.4^{b}**		26.6		3408.0	
	(4.2)		(103.2)		(185.0)		(2.5)		(4.6)		(414.9)		(79.4)		(36.4)		(341.5)		**(12.1)**		**(5.2)**		**(109.8)**		(16.2)		(1255.6)	
	56.8		3.6		21.8		0.3		0.5		4.8		0.2		1.0		98.7		39.0		22.9		141.7		4.4		**1406.7^{AB}**	
	(9.0)		(2.9)		(10.1)		(0.4)		(0.4)		(2.6)		(0.2)		(0.7)		(52.7)		(13.7)		(3.1)		(24.4)		(0.3)		**(496.9)**	

AryS = arylsulfatase activity; Chit = chitinase activity; Leu = leucine-aminopeptidase activity; Uroni = glucuronidase activity; α-Gluco = α-glucosidase activity; β-Gluco = β-glucosidase activity; Cell = cellulase activity; Xylo = xylosidase activity; AcPME = acid phosphomonoesterase activity; BisP = phosphodiesterase activity; PyroP = pyrophosphatase-phosphodiesterase activity; AlkPME = alkaline phosphomonoesterase activity; Inosit = inositol-phosphatase activity; Ester = acetate-esterase activity.
Data with statistically significant differences are highlighted using bold text.

Table 5
Soil enzyme activities normalized per unit of organic C of the organic (O) and mineral (A) horizons at the micro- (horizon), meso- (altitude), and macro- (latitude) scale for soils under European beech (*Fagus sylvatica* L.) forest from central Apennines (Italy) within 1° of latitude. The numbers in parentheses are the standard deviations. Within each variable (horizons, altitude, and latitude), column with different letters have means values that significantly differ for $P \leq 0.05$ for the Tukey's post-hoc test; for the altitude and latitude variables, lowercase letters refer to O horizons, capital letters to A horizons.

	AryS		Chit	Leu		Uroni		α-Gluco		β-Gluco		Cell		Xylo		AcPME		BisP		PyroP		AlkPME		Inosit		Ester	
	O	A	O	A	O	A	O	A	O	A	O	A	O	A	O	A	O	A	O	A	O	A	O	A	O	A	O	A
	nanomoles of 4-methylumbelliferone (7-amino-4-methyl coumarine) • g^{-1} organic C • $hour^{-1}$																											
Horizons																												
OLn	**15.7^{d}**		**428.4^{a}**	**753.8^{a}**		**10.3^{a}**		**21.0^{a}**		**1222.4^{a}**		**188.9^{a}**		**124.5^{a}**		**885.0^{ab}**		**101.8^{b}**		**37.9^{c}**		**767.5^{b}**		**70.8^{a}**		**7421.0^{a}**	
	(8.9)		**(126.5)**	**(286.5)**		**(3.1)**		**(4.9)**		**(596.5)**		**(123.7)**		**(46.0)**		**(739.5)**		**(24.4)**		**(23.6)**		**(319.7)**		**(17.1)**		**(1495.7)**	
OLv	**27.6^{c}**		**246.7^{b}**	**761.7^{a}**		**8.1^{ab}**		**15.9^{a}**		**441.2^{b}**		**65.5^{b}**		**60.5^{b}**		**572.9^{bc}**		**94.7^{b}**		**75.3^{b}**		**785.8^{b}**		**51.6^{b}**		**6445.3^{a}**	
	(21.1)		**(104.2)**	**(442.1)**		**(5.1)**		**(6.9)**		**(357.1)**		**(51.7)**		**(28.5)**		**(208.4)**		**(32.6)**		**(59.3)**		**(354.2)**		**(23.4)**		**(2089.2)**	
OH	**75.3^{b}**		**117.4^{c}**	**236.0^{b}**		**3.9^{bc}**		**4.8^{b}**		**78.5^{c}**		**7.9^{c}**		**15.9^{c}**		**397.9^{c}**		**96.8^{b}**		**125.2^{b}**		**864.5^{b}**		**23.1^{c}**		**4026.4^{b}**	
	(62.1)		**(53.6)**	**(89.6)**		**(3.3)**		**(2.7)**		**(53.4)**		**(5.9)**		**(9.2)**		**(158.2)**		**(47.0)**		**(112.5)**		**(427.3)**		**(9.7)**		**(1686.3)**	
A	**929.7^{a}**		**67.9^{c}**	**328.8^{b}**		**4.6^{c}**		**7.9^{b}**		**64.0^{c}**		**2.6^{c}**		**14.1^{c}**		**1358.4^{a}**		**574.0^{a}**		**430.2^{a}**		**2137.6^{a}**		**65.9^{ab}**		**21973.5^{c}**	
	(311.7)		**(58.6)**	**(142.3)**		**(5.1)**		**(5.5)**		**(39.1)**		**(2.3)**		**(10.7)**		**(928.5)**		**(195.6)**		**(159.8)**		**(597.8)**		**(22.9)**		**(15083.4)**	
Altitude																												
800 m	33.7		301.5	673.4		8.7		15.4		737.8		106.4		82.5		766.0		102.7		78.4		795.7		58.9		6701.6	
	(41.4)		(161.1)	(326.4)		(4.0)		(7.4)		(688.5)		(108.4)		(57.0)		(687.0)		(35.1)		(92.0)		(419.4)		(27.3)		(2062.2)	
	1111.0^{A}		**104.8^{A}**	**426.2^{A}**		**8.4^{A}**		**11.6^{A}**		**88.9^{A}**		**4.0^{A}**		**21.8^{A}**		**1884.2^{A}**		556.3		503.6		2363.6		**78.1^{A}**		**31842.5^{A}**	
	(280.6)		**(63.7)**	**(125.9)**		**(4.3)**		**(4.2)**		**(41.5)**		**(2.3)**		**(9.9)**		**(1088.4)**		(192.5)		(199.1)		(644.1)		**(17.3)**		**(15158.9)**	
1000 m	36.4		263.6	581.2		7.1		14.7		549.1		88.3		64.2		526.4		93.0		69.1		801.6		44.4		5711.3	
	(40.9)		(164.1)	(452.9)		(5.1)		(9.3)		(577.8)		(114.3)		(52.4)		(157.6)		(31.5)		(53.1)		(281.8)		(23.0)		(2270.1)	
	748.4^{B}		**31.0^{B}**	**231.4^{B}**		**0.9^{B}**		**4.3^{B}**		**39.1^{B}**		**1.1^{B}**		**6.4^{B}**		**832.6^{B}**		591.8		356.7		1911.6		**53.6^{B}**		**12104.5^{B}**	
	(236.9)		**(15.6)**	**(77.1)**		**(2.1)**		**(4.0)**		**(12.6)**		**(1.3)**		**(3.5)**		**(220.0)**		(215.3)		(59.7)		(498.8)		**(22.1)**		**(6080.8)**	
Latitude																												
Mount	56.5		255.3	728.1		6.6		14.2		524.7		71.4		56.3		533.1		**119.9^{a}**		**120.7^{a}**		**1013.2^{a}**		49.2		5406.5	
Terminillo	(58.8)		(143.1)	(517.9)		(5.2)		(9.9)		(551.4)		(76.1)		(48.1)		(136.2)		**(29.7)**		**(96.4)**		**(321.4)**		(23.8)		(2122.3)	
	931.0		92.6	254.5		4.6		5.1		46.9		2.0		8.1		978.5		591.9		529.6		2036.9		52.2		10430.7	
	(216.6)		(87.8)	(117.8)		(5.3)		(4.4)		(20.7)		(3.0)		(6.7)		(408.5)		(81.8)		(249.1)		(758.0)		(20.7)		(6321.9)	
Mount	29.4		237.1	540.3		7.7		15.3		408.7		62.4		69.2		570.0		**98.1^{ab}**		**70.2^{a}**		**805.1^{ab}**		53.6		6460.5	
San Vicino	(18.0)		(97.5)	(178.8)		(3.4)		(5.6)		(252.5)		(43.5)		(28.4)		(110.2)		**(28.9)**		**(48.3)**		**(388.9)**		(22.6)		(1589.1)	
	796.9		42.9	329.6		3.8		9.9		54.1		2.9		15.1		1164.8		436.4		348.1		1853.8		63.7		28613.5	
	(316.8)		(11.5)	(21.1)		(4.7)		(2.7)		(16.0)		(0.9)		(5.2)		(454.8)		(221.7)		(62.8)		(618.9)		(19.9)		(17949.0)	
Mount	17.4		340.0	584.5		9.3		15.7		918.7		146.6		93.1		810.1		**75.7^{b}**		**29.3^{b}**		**579.7^{b}**		52.8		6837.1	
Acuto	(9.7)		(201.7)	(352.2)		(4.7)		(8.5)		(803.0)		(151.0)		(69.6)		(803.4)		**(24.7)**		**(11.2)**		**(214.6)**		(31.4)		(2499.9)	
	1061.3		68.2	402.2		5.6		8.9		91.0		2.9		19.2		1931.9		693.8		412.9		2522.1		81.6		26876.2	
	(403.0)		(55.9)	(213.1)		(6.5)		(8.2)		(58.6)		(3.0)		(16.2)		(1451.3)		(199.1)		(71.2)		(186.4)		(22.5)		(14268.3)	

AryS = arylsulfatase activity; Chit = chitinase activity; Leu = leucine-aminopeptidase activity; Uroni = glucuronidase activity; α-Gluco = α-glucosidase activity; β-Gluco = β-glucosidase activity; Cell = cellulase activity; Xylo = xylosidase activity; AcPME = acid phosphomonoesterase activity; BisP = phosphodiesterase activity; PyroP = pyrophosphatase-phosphodiesterase activity; AlkPME = alkaline phosphomonoesterase activity; Inosit = inositol-phosphatase activity; Ester = acetate-esterase activity.
Data with statistically significant differences are highlighted using bold text.

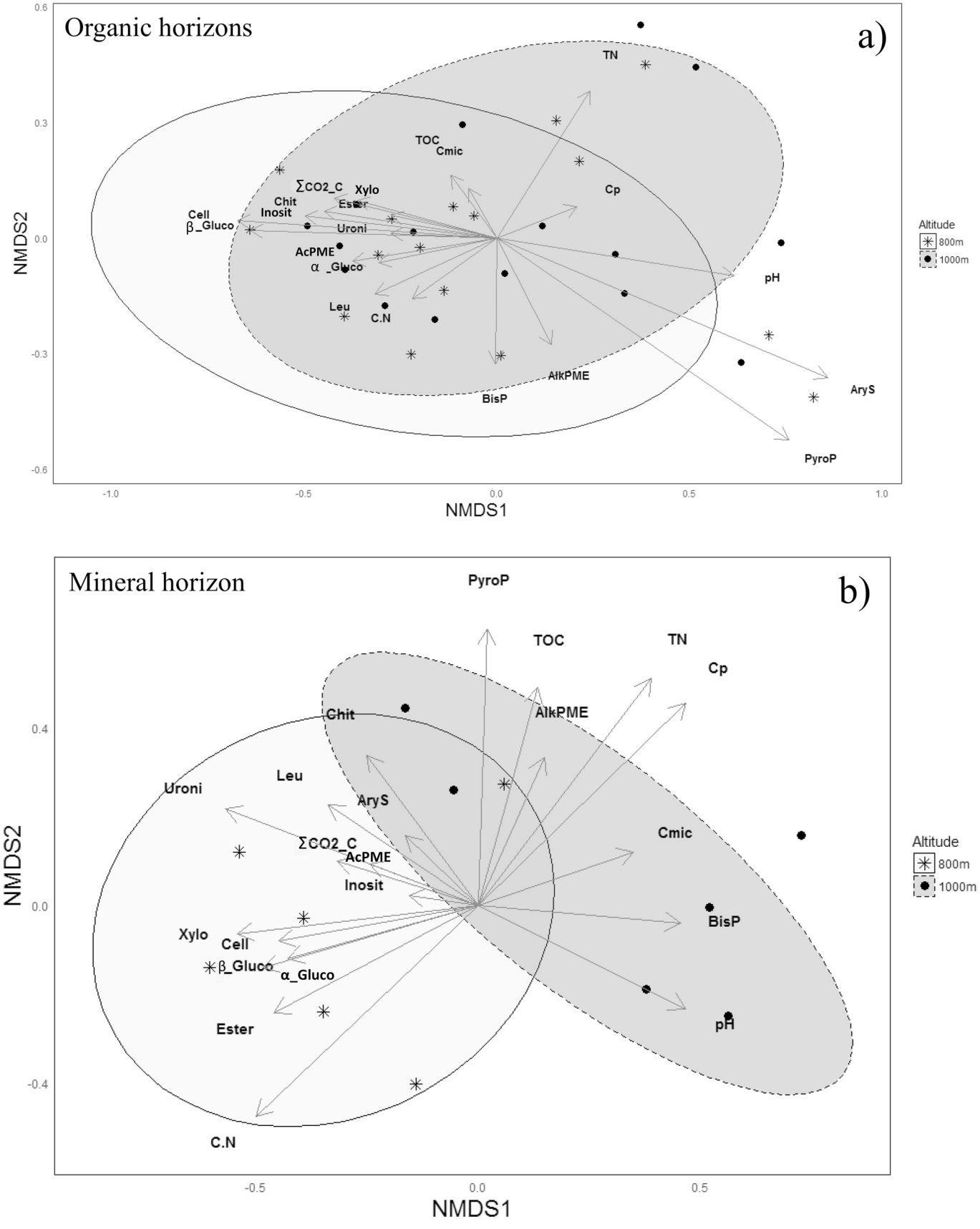

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Fig. 3. Variation of organic (a) and mineral (b) horizons topsoil chemical and biochemical properties as obtained by the NMDS analysis according to altitude. Chemical and biochemical properties: ΣCO_2–C = amount of CO_2 evolved during basal respiration experiments; C/N = C/N ratio; Cmic = microbial biomass carbon; C_p = Na-pyrophosphate extractable organic C; TN = total N; TOC = total organic C. Enzymes activities: AcPME = acid phosphomonoesterase activity; AlkPME = alkaline phosphomonoesterase activity; AryS = arylsulfatase activity; BisP = phosphodiesterase activity; Cell = cellulase activity; Chit = chitinase activity; Ester = acetate-esterase activity; Inosit = inositol-phosphatase activity; Leu = leucine-aminopeptidase activity; PyroP = pyrophosphatase-phosphodiesterase activity; Uroni = glucuronidase activity; Xylo = xylosidase activity; α-Gluco = α-glucosidase activity; β-Gluco = β-glucosidase activity.

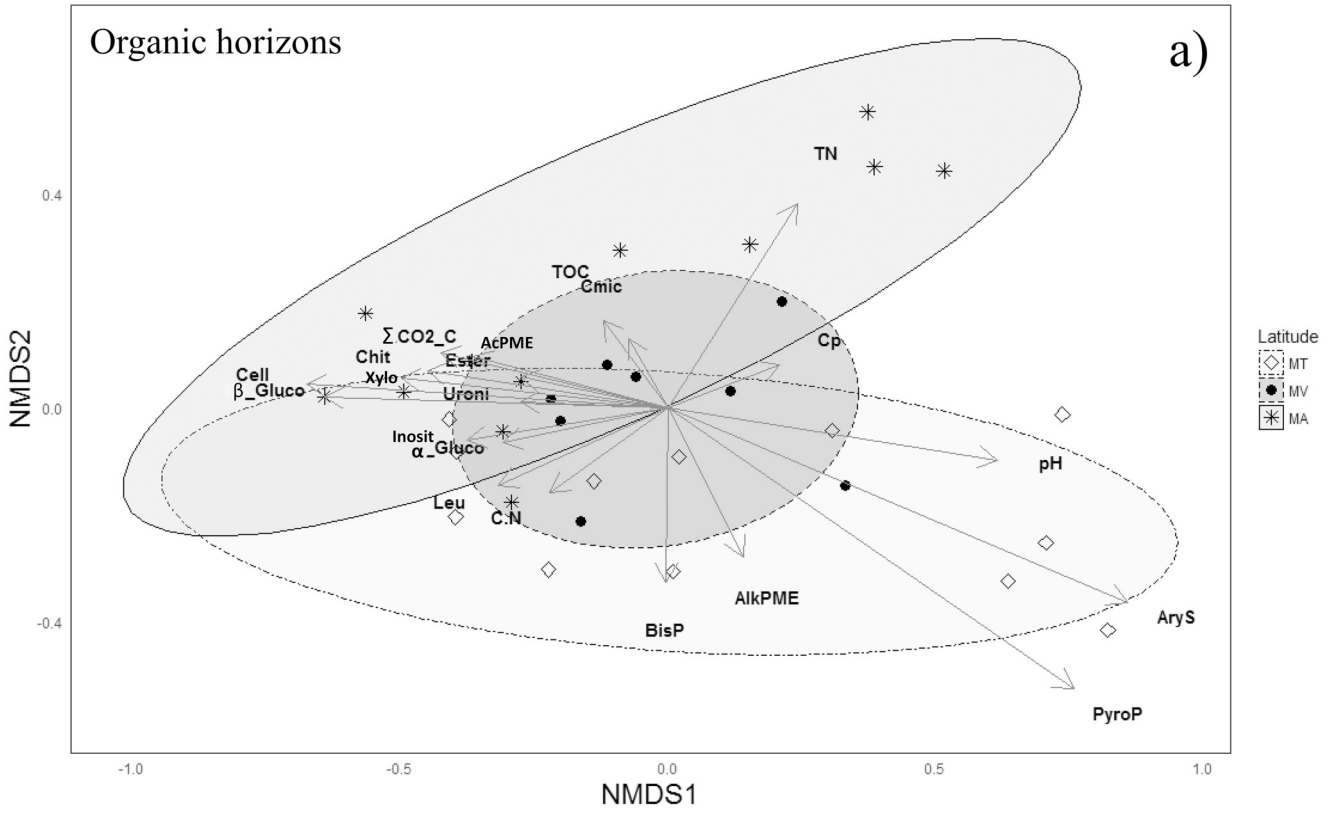
includes SOM that spans from fresh organic debris to low molecular weight organics derived from the activity of meso- and microfauna, and microbial communities (Schröder, 2008). Thus, it was expected that a decrease in TOC content would correspond to an increase in C_p content, even if the trend of this latter was not statistically significant for the O horizons. However, the contribution of C_p to TOC increased from OLn (5.0%) to OLv (6.0%), to OH (8.1%) and, much more, to A (22.2%) horizons. In parallel with the TOC content, the Cmic content and the ΣCO_2–C were the highest in the horizons made of poorly degraded vegetal debris (OLn and OLv), and decreased with depth, indicating that a high content of organic debris stimulates the activity of the microbial population responsible for the initial organic decay. In support of this, the enzymes involved in the hydrolysis of organic compounds (chitinase, leucine-aminopeptidase, glucuronidase, α-glucosidase, β-glucosidase, cellulase, xylosidase, acid phosphomonoesterase, inositol-phosphatase, and acetate-esterase) showed high activity (expressed on both soil mass and organic C bases) in the OLn/OLv horizons (in all the O horizons for alkaline phosphomonoesterase) and decreased with depth, in parallel with the increased decay of organics. Contrariwise, the activities of arylsulfatase and pyrophosphatase-phosphodiesterase, which are involved in advanced stages of SOM degradation, increased with depth. Our results partially agreed with those of many authors (e.g., Fontaine et al., 2007; Herold et al., 2014; Olander and Vitousek, 2000; Saiya-Cork et al., 2002), who found that most enzyme activities decrease with depth because of reduced fresh C input into deeper horizons. In support of our findings, the decreasing trend with depth of the C/N ratio indicated the presence of a relatively more degraded (humified) SOM in the deeper horizons. Given the NMDS results (Fig. 2), the general direction of TOC and C/N ratio vectors was similar to that of α-glucosidase, β-glucosidase, cellulase, xylosidase, and glucuronidase activities. These enzymes are involved in the degradation of cellulose, hemicellulose, pectins, and lignin, which are the main components of leaves and wood. Further, chitinase and leucine-aminopeptidase activities were in the same general direction of TOC and C/N ratio, possibly because they enhance their activity with the addition of fresh SOM (Kjøller and Struwe, 2002). Consequently, due to the input of fresh organic debris and the presence of macro- and mesofauna, the activities of these two enzymes were the highest in the OLn horizons and decreased in the A horizons. Among the enzyme responsible for P mineralization, acid phosphomonoesterase, phosphodiesterase, alkaline phosphomonoesterase, and inositol-phosphatase were more active in the O than in the A horizons. As inositol-phosphates are components of plant tissues (Turner et al., 2002a), it is possible that the input of vegetal debris in OLn horizons led to the stimulation of inositol-phosphatase activity in these horizons. The activity of phosphodiesterase on P-diesters evidently favored the release of P-monoesters and stimulated the acid and alkaline phosphomonoesterase activities (Hou et al., 2015; Nannipieri et al., 2011).

In the NMDS graph (Fig. 2), pH, arylsulfatase activity and pyrophosphatase-phosphodiesterase activity showed an opposite trend with respect to most chemical and biochemical properties. Contrary to the observations reported by Deng and Tabatabai (1997) for mineral horizons, in the organic horizons of the present study, arylsulfatase activity and pH were positively correlated (R^2 = 0.400) (Fig. 5a), and consequently increased with depth (Table 3). Thus, arylsulfatase activity was the highest in the A horizon, although the mean pH of the horizon (7.18) was outside the optimum pH range of this enzyme (5.5–6.2) (Tabatabai and Bremner, 1970). Several studies reported that the activity of arylsulfatase is correlated with SOM content (e.g., Dick et al., 1988; Tabatabai, 1994; Taylor et al., 2002) but in our organic horizons an opposite trend between arylsulfatase activity and TOC content was observed (Fig. 5b). However, it may be possible that arylsulfatase activity increases when SOM reaches advanced decay stages, as suggested by its positive correlation, although scarcely significant (R^2 = 0.062), with C_p (Fig. 5c). This hypothesis was supported by the highest arylsulfatase activity detected in the horizons with the major proportions of decayed SOM (OH and A horizons), where the microbial biomass was the lowest; as a matter of fact, the arylsulfatase activity was negatively correlated with Cmic (Fig. 5d). As a support to our findings, Prietzel (2001) reported that arylsulfatase activity increased in the organic horizons containing the highest humus and ester sulfate concentrations, and this was probably due to microbes that release S by mineralization of S-bearing compounds bound to the humic fraction by using organic C as source of energy (Solomon et al., 2001). For the pyrophosphatase-phosphodiesterase, Tabatabai and Dick (1979) reported that its activity in mineral soils is high in the upper horizons and decreases with depth due to the significant drop of SOM content. In the present case, pyrophosphatase-phosphodiesterase activity increased from OLn to OH if expressed on a soil mass basis, but it markedly increased from OLn to A horizon when expressed per organic C unit. The increasing pyrophosphatase-phosphodiesterase activity from the OLn horizon downward, other than being positively correlated with pH (Fig. 6a), was likely due to the decay level of SOM. In fact, as for the arylsulfatase activity, the pyrophosphatase-phosphodiesterase activity was negatively correlated with TOC content and positively correlated with Cp (Fig. 6b and c). The slight negative correlation between the pyrophosphatase-phosphodiesterase activity and Cmic (Fig. 6d) could be due to a possible partial inactivation of the enzyme due to the formation of organic colloid-enzyme complexes (Parent and MacKenzie, 1985).

In addition to pyrophosphatase-phosphodiesterase activity, three other P-cycle related enzyme activities (acid phosphomonoesterase, phosphodiesterase, and alkaline phosphomonoesterase) showed the highest activity in A horizons when normalized per unit of organic C. This behavior was ascribed to the major SOM decaying in the A horizons (with consequent greater availability of nucleic acids, carbohydrates, proteins, and fatty acids), where P-molecules were more available than in the O horizons and, consequently, stimulated the activity of these P-cycle related enzymes (Allison and Vitousek, 2005; Nannipieri et al., 2011).

4.2. Mesoscale level: altitude effect

The investigated soils of the sites at 800 and 1000 m a.s.l. (temperature difference of 1 °C), showed few dissimilarities in O horizons and much more in mineral horizons (Fig. 3a and b). In the O horizons, differences occurred for TOC and TN contents (Table 3), which were larger at 1000 than at 800 m and were attributed to lower microbial activity due to colder temperatures of sites at higher altitude (Blume et al., 2002; Xu et al., 2014). In fact, colder soil temperatures allow greater accumulation of SOM (Garten and Hanson, 2006; Tsui et al., 2013), as shown for the environments here investigated by De Feudis et al. (2016). In the A horizons, while TN, Cp and Cmic contents increased, the activities of the whole enzymatic pool decreased with increasing altitude when expressed per organic C unit; the only exception being three P-cycling related enzyme activities (phosphodiesterase,



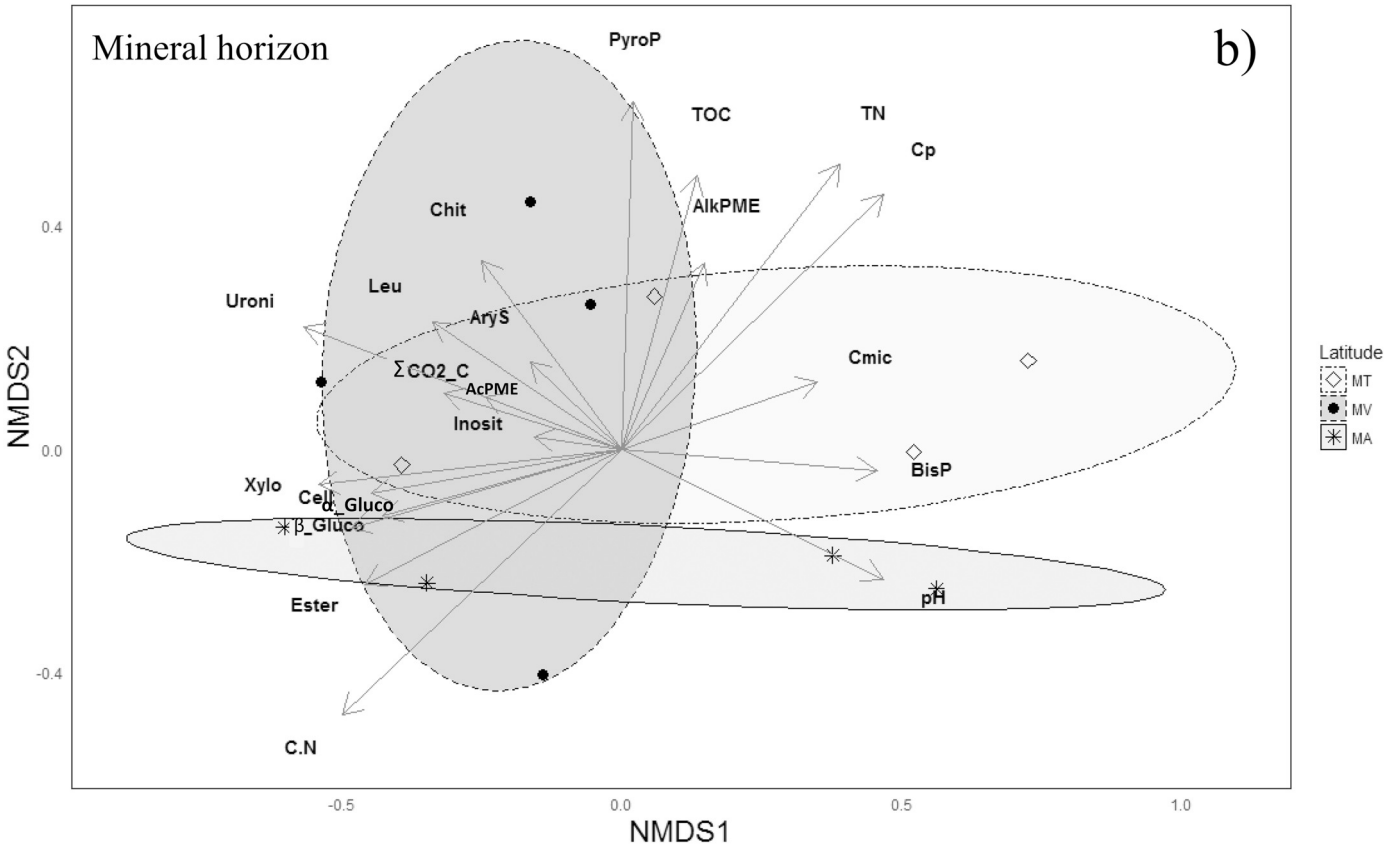


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Fig. 4. Variation of organic (a) and mineral (b) horizons topsoil chemical and biochemical properties as obtained by the NMDS analysis according to latitude. Chemical and biochemical properties: ΣCO_2–C = amount of CO_2 evolved during basal respiration experiments; C/N = C/N ratio; Cmic = microbial biomass carbon; C_p = Na-pyrophosphate extractable organic C; TN = total N; TOC = total organic C. Enzymes activities: AcPME = acid phosphomonoesterase activity; AlkPME = alkaline phosphomonoesterase activity; AryS = arylsulfatase activity; BisP = phosphodiesterase activity; Cell = cellulase activity; Chit = chitinase activity; Ester = acetate-esterase activity; Inosit = inositol-phosphatase activity; Leu = leucine-aminopeptidase activity; PyroP = pyrophosphatase-phosphodiesterase activity; Uroni = glucuronidase activity; Xylo = xylosidase activity; α-Gluco = α-glucosidase activity; β-Gluco = β-glucosidase activity.

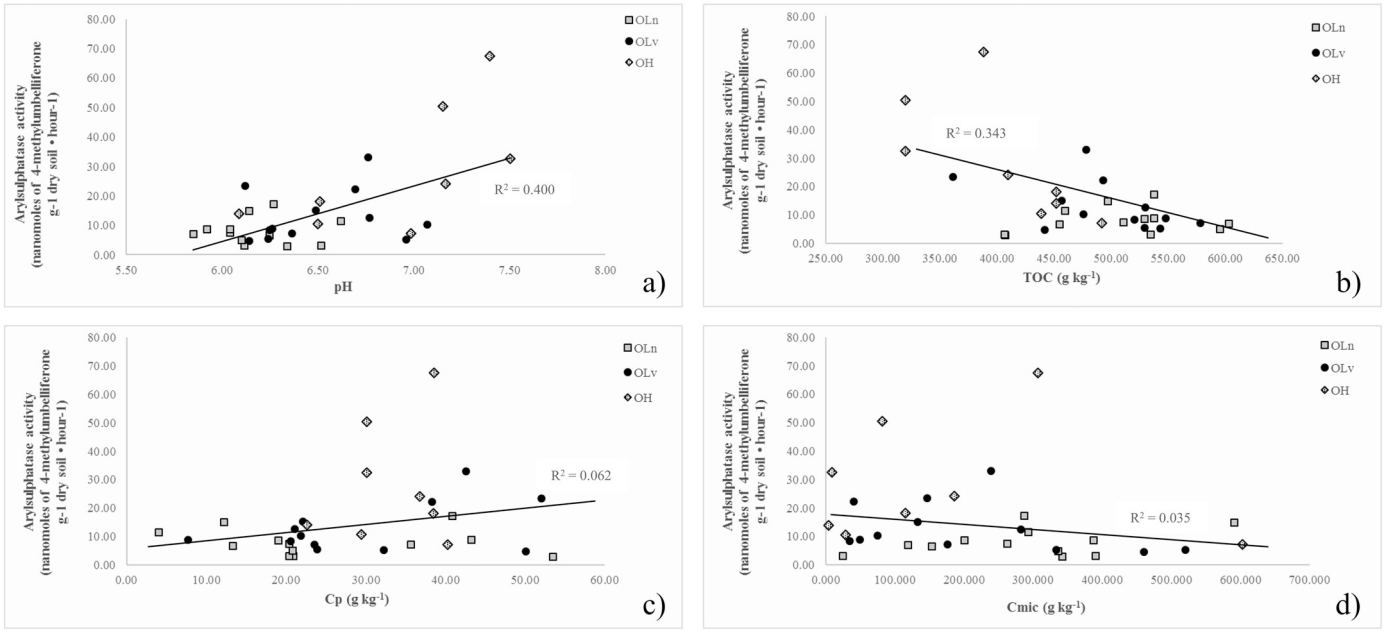

Fig. 5. Linear correlation between (a) arylsulfatase activity and pH, (b) arylsulfatase activity and TOC, (c) arylsulfatase activity and extractable organic C (C_p), and (d) arylsulfatase activity and microbial biomass carbon (Cmic) for the organic horizons.

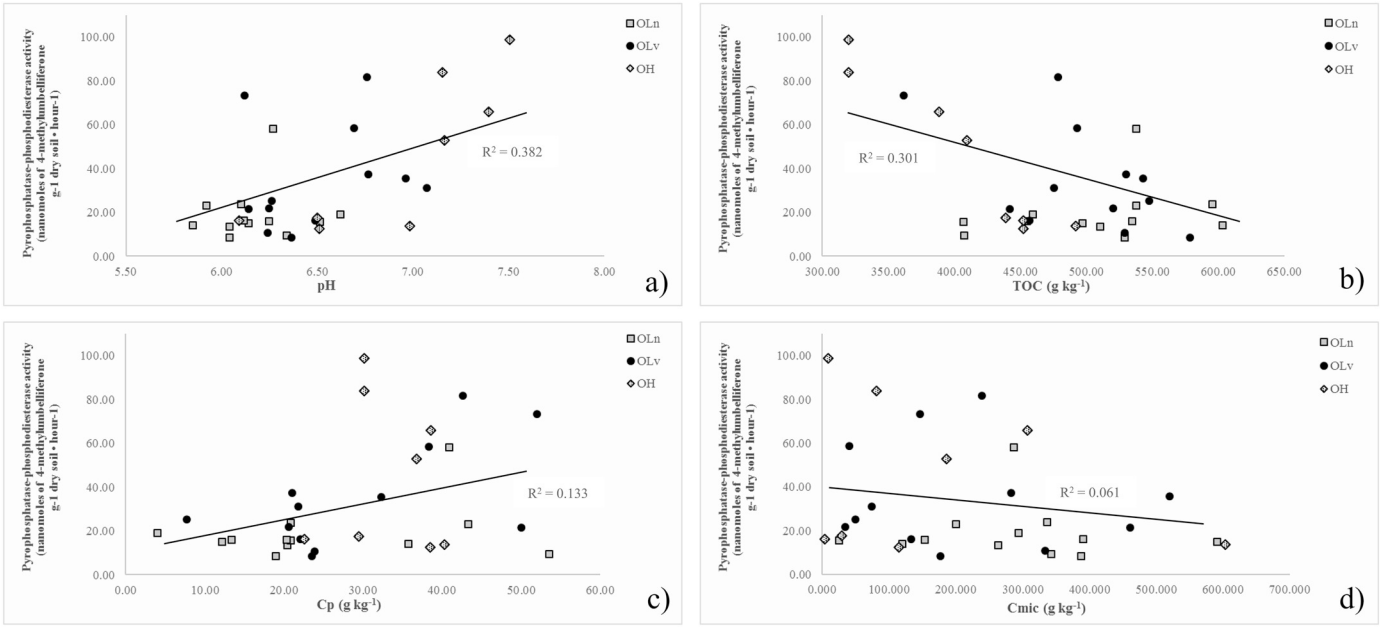

Fig. 6. Linear correlation between (a) pyrophosphatase-phosphodiesterase activity and pH, (b) pyrophosphatase-phosphodiesterase activity and TOC, (c) pyrophosphatase-phosphodiesterase activity and extractable organic C (C_p), and (d) pyrophosphatase-phosphodiesterase activity and microbial biomass carbon (Cmic) for the organic horizons.

pyrophosphatase-phosphodiesterase and alkaline phosphomonoesterase). Lower enzyme activity per organic C unit in A horizons at 1000 m was ascribed to higher TN and Cp content because, as already observed at microscale level, the enzyme activities involved in the hydrolysis of organic compounds decreased with increased organic decay. However, colder temperatures at higher altitudes particularly during winter (mean winter temperature was 1.4 °C at 800 m and 0.5 °C at 1000 m) probably had an effect. In fact, different from the O horizons, the A horizons in winter are often close to water-saturation. Consequently, they can more easily conduct cold temperature and

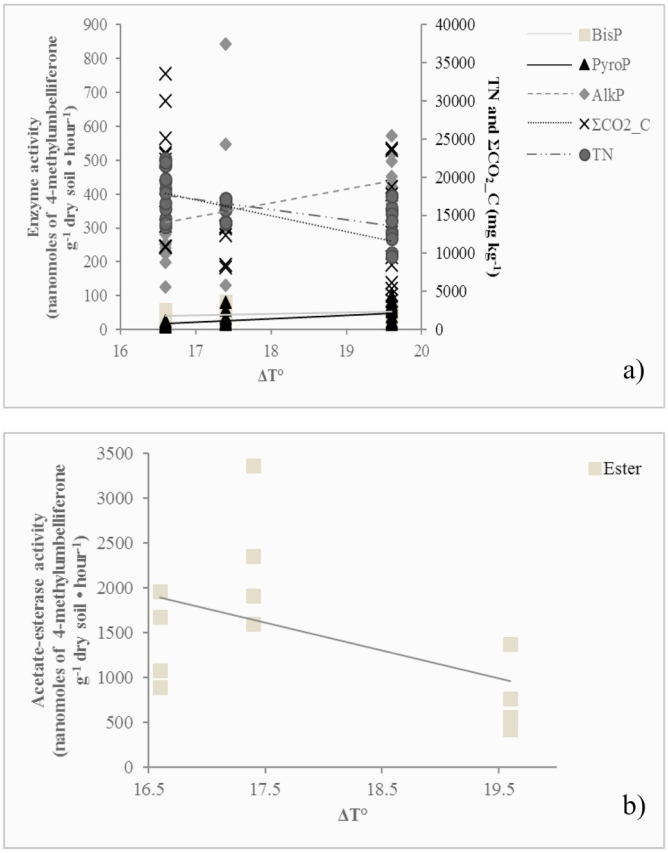

Fig. 7. Correlation between mean summer-winter thermal excursion and (a) ΣCO_2–C = basal respiration, AlkPME = alkaline phosphomonoesterase activity; BisP = phosphodiesterase activity, PyroP = pyrophosphatase-phosphodiesterase activity, and, TN = total nitrogen and (b) Ester = acetate-esterase activity.

Table 6
Mean summer air temperature (MSAT), mean winter air temperature (MWAT), and summer-winter thermal excursion (ΔT) for the areas at 800 and 1000 m of altitude, and mean of the thermal excursions at 800 and 1000 m of altitude (MΔT) for soils under European beech (*Fagus sylvatica* L.) forest from central Apennines (Italy) within 1° of latitude.

	800 m a.s.l.			1000 m a.s.l.			MΔT
	MSAT	MWAT	ΔT	MSAT	MWAT	ΔT	
	°C						
Mount Terminillo	18.9	2.1	16.8	17.7	1.3	16.4	16.6
Mount San Vicino	19.1	1.8	17.3	18.1	0.8	17.3	17.3
Mount Acuto	20.1	0.4	19.7	19.0	−0.5	19.5	19.6

Table 7
R^2 values of correlations between soil properties and the mean summer-winter thermal excursion.

Soil property	R^2 value
BisP	0.166
PyroP	0.284
AlkP	0.153
Ester	0.241
TN	0.323
ΣCO_2–C	0.141

eventually freeze. This happens especially when the thickness of organic horizons and/or a snow mantle is scarce (generally < 40 cm) limiting thermal insulation of mineral horizons (Maksimova et al., 1977; Mackiewicz, 2012; Allegrezza et al., 2017; Cocco et al., 2018). This would also indicate that most of the tested enzyme activities are rather sensitive to temperature. For example, β-glucosidase activity is known to be sensitive to temperature (German et al., 2012) and is considered an important parameter for soil quality monitoring due to its role in SOM turnover (Turner et al., 2002b; Stott et al., 2010; Nannipieri et al., 2012). In contrast, the fact that three of four P-cycle related enzyme activities (phosphodiesterase, pyrophosphatase-phosphodiesterase, and alkaline phosphomonoesterase) showed similar values at both altitudes supports the hypothesis that their activities are mostly controlled by TOC content (Weintraub et al., 2012; Zhang et al., 2015). However, in spite of the higher TOC content in the organic horizons at 1000 than at 800 m, we did not observe a significant difference of the topsoil thickness. Even though this can be ascribed to the small temperature gradient (1 °C) and the small number of profiles trenched, topsoil thickness is also affected by factors like biomass production, wind-blowing intensity, soil faunal perturbation, and slope dynamics (Agnelli et al., 2016).

4.3. Macroscale level: latitude effect

The NMDS analysis indicated that chemical and biological differences of the soils at MA, MV, and MT were few (Fig. 4a and b), probably because of their similar environmental conditions. For this reason, none of the environmental factors taken into consideration was able to explain the differences among locations that occurred for TN content, ΣCO_2–C, and enzyme activities expressed both on a soil mass basis and per organic C unit. In particular, from south to north the O horizons showed an increasing trend of TN and ΣCO_2–C, while enzyme activities involved in the P-cycle decreased (Fig. 7a); on the contrary, in the mineral horizons the esterase activity tended to increase (Fig. 7b). As MAAT was the same at the three locations, mean seasonal air temperature was considered. From the analysis of the mean monthly temperatures, the northernmost location (MA) was characterized by the lowest winter value and the highest summer value, while the southernmost location (MT) had milder winters and cooler summers than the other locations (Table 6). Therefore, while the south-to-north MAAT gradient was constant, the summer-winter temperature excursion increased 3 °C at both 800 and 1000 m altitudes. Because of this, an effect of thermal excursion on soil properties along the latitudinal transect was investigated. Correlations between the enzyme activities and TN and ΣCO_2–C that differed among locations and the mean summer-winter thermal excursion of each location (Fig. 7) were all significant, with R^2 values varying from 0.14 to 0.32 (Table 7). Change to temperature parameters likely indirectly affects soil properties by modifying the amount of available substrate and the microbial nutrient demand and activity (Baldrian et al., 2013). In this sense, a wider thermal excursion corresponds to more intense stress conditions for plants and microorganisms (e.g., Stevenson et al., 2014). Under these conditions, macro- and mesofauna and microbial communities are fostered to degrade SOM for energetic purposes (Allison et al., 2007), so increasing availability of phosphates and reducing enzyme activity. This likely happens more in the O than in the A horizons since the upper horizons feature maximum thermal excursion.

5. Conclusions

This study has established that horizons, altitude, and latitude affect the chemical and biochemical properties of the topsoil of beech forests at different extents. At the microscale level (soil horizons), a well-

defined gradient of organic degradation was responsible for the differentiation of OLn, OLv, OH, and A horizons. Layering was consistent with the chemical and biochemical properties of the horizons. At the mesoscale level (altitude), the difference of 1 °C in MAAT appeared to affect few of the tested chemical and biochemical properties, and mainly in the A horizons. At the macroscale level, locations within a small latitudinal transect (1°) and with similar MAAT featured soils showing even fewer differences, and mostly in the O horizons; in this case, the summer-winter thermal excursion likely had an effect on the plant-soil system and on the activity of macrofauna, mesofauna, and microbial communities and on enzyme activities. Considering all the investigated aspects, in a perspective of warming climate, a temperature rise of 1 °C (in the MAAT range of 9–10 °C) might lead to an increase at the higher altitudes of microbial communities and enzyme activities sensitive to temperature. Consequently, more intense degradation of organic debris and a re-arrangement of the forest floor may occur. In sites at 1000 m a.s.l., minor changes in TOC and TN are possible (relative to sites at 800 m) but with no substantial differences in the thickness of topsoil or single O and A horizons. Because of this, since litter degradation is considered a direct source of CO_2 to the atmosphere, the consequence of its enhanced decomposition rate in temperate beech forests may affect actual soil-plant equilibria regulating the concentration of atmospheric CO_2. Further studies of latitudinal/altitudinal gradients are justified and encouraged such that changes in chemical and biochemical soil properties across such are better understood.

Availability of data and materials

Rough data are available on request to the authors.

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Compliance with ethical standards

The authors declare that they have no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.geoderma.2019.01.043.

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